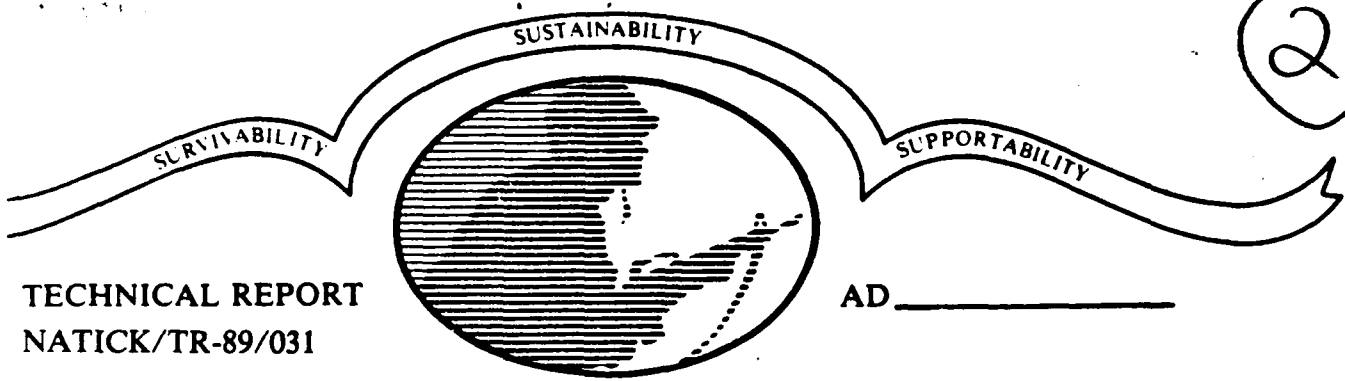


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TECHNICAL REPORT
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EFFECT OF HALOGEN-TREATED WATER ON MICRONUTRIENTS IN MILITARY RATION BEVERAGES

BY

BONITA M. ATWOOD
C. PATRICK DUNNE

MAY 1989

FINAL REPORT

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<p>→ Halogen-micronutrient interactions in reconstituted military beverages were measured. Effects of halogen on micronutrient content and vice versa were determined. Halogen in purified water is completely destroyed upon mixing with standard beverage bases, whether the resulting beverage is acid or neutral. Both fortified and unfortified beverages have this effect--due to reactivity of beverage components, especially flavors and colors.</p> <p>The most reactive vitamin tested was ascorbic acid, which instantaneously removed all halogen from purified water. Even encapsulation did not completely counteract this effect.</p> <p>It was concluded that the effect of halogenating agent on micronutrient content of reconstituted beverages was negligible due to the presence of extremely reactive substances in beverage formulations and the large excess of ascorbic acid in most beverages. Commercially available encapsulation of vitamins will not protect halogens from reaction. Strict adherence to military field recommendations for water purification is required for microbiological safety when treated water is added to the beverage bases now in rations. <i>Keywords:</i></p>					
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PREFACE

Previous work has demonstrated that commercial beverage powders exert a great halogen demand on the residual halogens in treated water. The present study deals with military ration beverage items and, in addition to determining their halogen demand, investigates the effect of added halogen on the micronutrient content of the reconstituted beverages. We studied acid and neutral beverages and the reactions of their ingredients with halogens to determine the effects of halogens on micronutrients. Encapsulation was considered as a means of reducing these effects, and initial evaluations of commercially available encapsulants were conducted.

The authors wish to thank Ms. Jennifer Ellms and Mr. James Fleury for their excellent help toward achieving the objectives of this study. Mr. Fleury, in particular, working as a student contractor (Contract No. DAAK60-86-M-1987/18 Apr 86), gave invaluable aid in the treatment of data collected in support of this project. The help of all persons involved in support of this work, in particular Mr. Jack Howker and SGT Geoff Phillips working in the Food Engineering Directorate (FED) and Mr. Morris Rogers working in the Science and Advanced Technology Directorate (SATD), is greatly appreciated.

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EFFECT OF HALOGEN-TREATED WATER ON MICRONUTRIENTS IN MILITARY RATION BEVERAGES

INTRODUCTION

Water purification of individual portions can be accomplished in the field by the use of iodine tablets. For purification on a larger scale calcium hypochlorite can be used. Both of these agents yield free available halogen to inactivate bacteria, cysts of amoeba, and viruses, which may be present in water found in the field.⁽¹⁻⁴⁾ In order for the halogen to be effective in purifying the water, it must be present in sufficient quantity for the length of time necessary for pathogen inactivation. The recommended level for iodine is 12 to 16 ppm and for chlorine is 4 to 5 ppm residual halogen. The effective halogen content may be reduced either by substances present in the water or by interaction with constituents of beverages reconstituted with the treated water.

Rogers et al.⁽⁵⁾ conducted a study to determine the halogen demand of commercial beverage powders. They also looked at the constituents of the beverages and beverage powders as well as vessels with which the beverages would come into contact. They found that commercial beverage formulations exert a high halogen demand.

This study seeks to expand the items tested beyond acidic fruit-flavored beverages and to include neutral beverages, such as cocoa. In addition, the relative reactivity of ingredients in appropriate buffers (acid or neutral) is determined. The effect of halogen on vitamin stability over time and temperature is also monitored. It seems reasonable that those components that react the fastest with the halogen

will be first removed from solution. However, without following all components over time, one cannot say with certainty that this is so. The principal components followed were vitamins as they reacted with the halogens iodine or chlorine.

By studying commercially available encapsulated vitamins and encapsulation agents, one can assess the efficacy and need for encapsulation to minimize halogen-micronutrient interactions.

MATERIALS

Orange beverage powder, cocoa beverage powder, and electrolyte drink mixes were obtained from the Food Engineering Directorate (FED), Natick RD&E Center. Detailed formulas are listed in the Appendix. Coffee was a commercial instant product purchased on the open market, not fortified with vitamin C, as is the instant coffee in the Meal, Ready-to-Eat (MRE).

Buffer solutions were prepared from reagent grade chemicals. For tests involving the reactions of orange beverage and cocoa ingredients, these ingredients were obtained from FED or from suppliers of reagent chemicals. Vitamins tested (thiamin hydrochloride, riboflavin, and ascorbic acid) were from Nutritional Biochemicals Corporation, Cleveland, Ohio. All the labile vitamins were stored in brown bottles in a refrigerator.

Encapsulated vitamin C was obtained from various suppliers to the food industry. These included Durkee Industrial Foods Group, SCM Corporation, Cleveland, Ohio; Desmo Chemical Corporation, St. Louis, Missouri; Balchem Corporation, Slate Hill, New York; and E. Merck, Darmstadt, Germany.

The halogens used were obtained through normal government supply channels. Iodine 50 water purification tablets are tetraglycine

hydroperiodide with disodium dihydrogen pyrophosphate added as excipient⁽³⁾ and are manufactured by Wisconsin Pharmacal, Milwaukee, Wisconsin. Tablets are compounded so that 1 tablet per liter yields a solution that is nominally 8 ppm in active iodine. Calcium hypochlorite was obtained in sealed glass ampoules, the quantity per ampoule being sufficient to sterilize one Lyster bag of water (36 gallons) in the field.

METHODS

A. Iodine

Iodine solutions were prepared fresh daily using water purification tablets. The water used was distilled or deionized through a Milli Q water system (Millipore Co.). The iodine content of the water was determined using a Wallace and Tiernan amperometric titrator following the method for total available iodine supplied with the titrator. A 200 mL aliquot of the solution being tested was placed in the agitator cup and 1 mL of pH 4.0 buffer and 1 mL of potassium iodide (KI) was added. This adjusted the pH of the solution to 4.0 - 4.5, which is necessary for iodine titrations. The solution was agitated and phenylarsene oxide (0.00564N) added until the deflection of the needle was either zero or much less than that observed on addition of the previous aliquot. For acid beverages this procedure presented no problem since pH of the solutions was near 4.0 - 4.5. For neutral beverages and buffered solutions of vitamins, 500 μ L glacial acetic acid had to be added to the sample before continuing with the procedure so the pH would be in the correct range.

B. Chlorine

A stock solution of calcium hypochlorite from freshly opened, sealed glass ampoules was prepared by dissolving one ampoule of calcium hypochlorite in one liter of distilled water. This stock solution was diluted 1 to 50 for experimental use. Stock solutions were kept for no more than five days before replacing with fresh stock. Chlorine content was determined using the Wallace and Tiernan amperometric titrator following the method for free available residual chlorine supplied with the titrator. The pH of a 200 mL aliquot of solution was adjusted to 7.0 by addition of 1 mL of pH 7.0 buffer. The procedure is then the same as for iodine with addition of phenylarsene oxide to the end point. The milliliters of titrant used are equivalent to the ppm of chlorine in solution.

C. Vitamins

Vitamin content of the various beverages was determined according to procedures outlined in Methods of Vitamin Assay, 3rd edition⁽⁶⁾ with modifications as necessary. Thiamin was determined by the thiochrome method. For determining thiamin in iodinated buffer solutions not containing vitamin C, it was found that iodine present in the solution interfered with the fluorescence determination. To eliminate this problem, a pinch of vitamin C (1 - 2 μ g) was added to each sample before the final dilution. This destroyed the residual iodine present, so fluorescence determinations could then be made.

Riboflavin was determined by the fluorometric method. Ascorbic acid was determined spectrophotometrically using a slight modification of the published procedure. An aliquot of the test solution was removed for

analysis and brought to volume with 3% metaphosphoric acid. Five mL of this was added to five mL of dichlorophenolindophenol dye solution in a cuvette and the galvanometer deflection of an Evelyn Colorimeter noted after 10 s and 20 s. The corrected colorimetric reading ($D_{10} - [D_{20} - D_{10}]$ where D_{10} and D_{20} are the colorimetric readings at 10 s and 20 s, respectively) was used to calculate concentration of vitamin C in the sample. This method corrects for any slower acting reducing agents in the sample compounds. A standard was run each day to establish an instrument constant.

EXPERIMENTAL

In order to be able to assess the necessity and feasibility of fortifying ration items with coated vitamins to minimize halogen-vitamin interactions, it is necessary to first determine what key interactions occur. To do this, various fortified drink mixes were reconstituted with halogenated water, then the content of specific vitamins was measured as a function of time and temperature. The halogen content was also determined under the same conditions. The reconstituted drinks are classified as either acid or neutral, as it was felt that different rates of reaction might be observed at different pH values.

In order to study interactions of selected beverage components with halogens, buffers of appropriate pH to emulate orange beverage and cocoa were prepared. The formulations for each of the buffers are given in Table 1. The acid buffer to simulate orange beverage had a pH of 3.5 - 3.8. The pH of the neutral buffer used to simulate cocoa was adjusted to 7.0 with sodium hydroxide, 15%.

Table 1. Buffer Formulations for Testing Ingredient-Halogen Interactions

<u>Acid (pH = 3.5 - 3.8)</u>		
<u>Component</u>	<u>Amount (g/L)</u>	<u>Molarity</u>
Citric Acid, Anhydrous	5.4	Total citrate = 31.1 mmolar
Tricalcium Phosphate	2.22	7.09 mmolar
Potassium Citrate Monohydrate	0.984	Incl. in total citrate

<u>Neutral (pH = 7.0)</u>		
<u>Component</u>	<u>Amount (mL/L)</u>	<u>Molarity</u>
Phosphoric Acid	1.28	12.5 mmolar
Sodium Hydroxide, 15%	8 (to pH 7)	

Various ingredients of orange beverage powder were tested for their effect on iodine. For this work the acid buffer was used. Cocoa mix ingredients were tested using the neutral buffer. Table 2 lists the various ingredients tested and the results of those tests.

Table 2. Reaction of Beverage Ingredients with Iodine
in Buffer at 25°C*

<u>INGREDIENT</u>	<u>pH</u>	<u>RESULT**</u>
Glucose	3.8	-
Sucrose	3.8	-
Orange flavor #22448	3.7	+
Orange flavoring #92251	3.8	+
Ascorbic acid	3.8	+
Thiamin	3.7	-
Cocoa	7.0	+
Cremora (R)§	7.0	+
Whey protein	7.0	+
Salt	7.0	-
Cane sugar	7.0	-
Vanilla	7.0	-

*Iodine present at 2 tablets/L or approx 16 ppm.

**Indicates little or no removal of iodine after 30 min compared to
buffer alone

+Indicates removal of iodine.

§ Cremora is a registered trade name. Citation of trade names in this
report does not constitute an official endorsement or approval of the
use of such products.

Ingredients were tested at levels corresponding to amounts per serving of beverage. The ingredients of the electrolyte drink mix were not separately tested, although most of the ingredients are the same as those in the orange beverage formulation. Since coffee is not a formulated product, separate ingredients could not be tested, although both fortified (containing added vitamin C) and unfortified (no added vitamin C) coffee were tested. The formulations for fortified orange beverage powder, electrolyte drink base, and fortified cocoa beverage powder are given in the Appendix.

Potential encapsulating agents were also screened for their reaction with iodinated water. Table 3 lists the agents tested and the results of the screening.

Additionally, several coated vitamin formulations were tested for interaction with iodine using the appropriate buffers. The formulations tested and the results are given in Table 4.

A. Acid Beverages

This category includes those beverages which, when reconstituted have a pH of less than 6 - 7. Beverages included are orange beverage, coffee, and electrolyte drinks.

1. Orange beverage. A weighed amount of fortified orange beverage powder (30 g/250 mL serving), obtained from FED as part of a production of beverage bars for Arctic Ration and Food Packet, Assault, was dissolved in the appropriate amount of water, while stirring on a magnetic stirrer. The initial aliquots were removed within two minutes for determination of halogen, thiamin, and/or ascorbic acid. The time of this withdrawal was arbitrarily assigned to be zero time for kinetic plots. Subsequent

Table 3. Testing of Iodine Reactivity of Potential Encapsulating Agents

<u>AGENT</u>	<u>SUPPLIER</u>	<u>CONC'N (MG/L)</u>	<u>RESULT*</u>
1. Maltrin-M-040 Maltodextrin	Grain Processing Corp. (GPC) Muscatin, Iowa	408	Purple complex forms
2. Maltrin-M-150 Maltodextrin	GPC	400.4	No change
3. Centrolex R Lecithin	Central Soya Chemurgy Division Gibson City, IL	405.6	Color disappears on heating
4. Apple Pectin	Pektin-Fabrik Hermann Herbstreith KG Nuenburg/Wurtt, FRG	403.2	Not much loss even with heating
5. Lecithin	Staley	not measured	Slight color loss
6. Maltrin-M-100 Maltodextrin	GPC	403.2	Slight purple color
7. Kacmel Hydrogenated Vegetable Oil	Durkee, SCM Corp. Cleveland, Ohio	404.8	No obvious color loss
8. Gelatin-USP	Fisher Scientific Fairlawn, NJ	410.8	Color loss on heating
9. Bacto-agar	Difco Laboratories Detroit, Michigan	417.6	Color loss on heating
10. Pullulan-30	Hayashibara Biochemical Labs, Inc.	200	Slight loss after 30 min
11. Egg Albumin	J. T. Baker Chemical Co. Phillipsburg, NJ	408	50% loss after 5 min

*Result is based on visual observation of the solutions. Concentration of iodine remaining was measured amperometrically in the case of pullulan 30 and of egg albumin. Iodine present at an initial concentration of 16 ppm.

Table 4. Reaction of Coated Vitamins with Iodine in Buffer

<u>VITAMIN</u>	<u>COATING</u>	<u>% VITAMIN</u>	<u>SUPPLIER</u>	<u>SOLUBILITY</u>	<u>REMARKS</u>	<u>pH</u>
C	Ethyl cellulose	97.5	Merck	Water	Reacted w/iodine quickly	3.8
C	Maltodextrin	70	Durkee	Water	Reacted w/iodine quickly	3.8
C	Partially hydrogenated palm oil	70	Balchem	Fat	Reacted w/iodine within 1 min	3.8
C	Partially hydrogenated soybean oil	70	Durkee	Fat	Reacted w/iodine quickly	3.8
C	Mono and diglycerides	60	Desmo	Fat	Reacted w/iodine quickly	3.8
C	Partially hydrogenated cottonseed oil	50	Durkee	Fat	Reaction w/iodine depends on integrity of capsules	7.0

aliquots were removed for assay of the mentioned components at intervals determined by expected decay of the component being measured. That is to say, a sample protocol was designed to cover at least one kinetic half-life of the reactive component. Additionally, a halogenated water control was run concurrently under the same conditions with aliquots being removed at the same time intervals as used for the reconstituted beverage powder. Determinations were run using one or more iodine tablets per liter, each tablet supplying approximately 8 ppm free iodine; calcium hypochlorite at a level of 5 ppm free chlorine; and, as a control for autoxidation of vitamins, distilled water. Experiments were carried out at room and elevated temperatures.

For work at elevated temperatures, all solutions were equilibrated at the temperature of interest and the entire determination run in a shaking water bath so agitation was possible. Agitation simulated a canteen on a walking soldier, and it ensured uniform mixing for removal of aliquots.

2. Coffee. Unfortified instant coffee was purchased commercially and used for all work with coffee--halogen interactions. Coffee present in military field rations is fortified with vitamin C at the level of 15 mg per 250 mL serving. Both fortified and unfortified coffee were examined in order to correct for readings imparted by color of the coffee solutions. For unfortified work 2.5 g coffee per 250 mL serving were used, and for fortification 15 mg vitamin C per 250 mL serving were added. The kinetic sampling procedure used was the same as for orange beverage, with only halogen and ascorbic acid determinations being made. Effects of iodine concentration and temperature were also measured. The pH of unfortified coffee was 4.58 and that of the fortified was 4.51.

3. Electrolyte drinks. Electrolyte drink mixes were obtained from FED. These included a beverage base as well as flavored mixes. Flavors supplied included apple, cherry, lemon, and lemon-lime. Each packet (28 g) was sufficient to prepare one quart of drink. None of the flavors or the base was fortified in any way. Consequently, only the change in halogen concentration was determined. This analysis was run at room temperature. Additionally, the effects of adding ascorbic acid to the base were determined; in those cases both iodine and ascorbic acid contents were followed. The pH of the beverage base in water was measured and found to be 2.9.

B. Neutral Beverages

These are beverages having a pH in the range 6 - 7. The only neutral beverage studied was cocoa, which for the MRE is fortified with ascorbic acid and thiamin, among others. In all cases, one packet of cocoa was reconstituted with 177 mL of the appropriate water, then thiamin, ascorbic acid and halogen contents determined at appropriate time intervals. The pH of reconstituted MRE cocoa was 6.5.

RESULTS AND DISCUSSION

A. Acid Beverages

1. Orange beverage. Orange beverage (OB) bars are a component of the Food Packet, Assault and of the Arctic Ration. Also, flavored beverage bars, similar to the orange bar, are a component of the Ration, Lightweight, 30 Day, which is under development by FED. The contribution of these and other fortified beverages to vitamin C nutrition can be seen from Table 5. Thus it is important to know whether the vitamin C content of the reconstituted beverage will be affected by the water used to reconstitute the beverage.

Table 5. Importance of Fortified Beverages to Vitamin Nutrition

<u>RATION</u>	<u>AVERAGE MG. VITAMIN C SUPPLIED PER DAY</u>	<u>% OF DAILY INTAKE SUPPLIED BY FORTIFIED BEVERAGES</u>	
FOOD PACKET, ASSAULT	76	ORANGE BEVERAGE COFFEE	68 20
ARCTIC RATION (1984 VERSION)	326	ORANGE BEVERAGE COFFEE COCOA POWDER	32 5 30
MEAL, READY-TO-EAT (1980 PROCUREMENT)	225	COCOA POWDER COFFEE	22 7
RATION, LIGHTWEIGHT, 30 DAY (IN DEVELOPMENT)	120	FLAVORED BEVERAGE	100

Most of the work with orange beverage was done using the 16 ppm level of iodine (2 tablets/liter). However, we also decided to determine if the level of iodine had any effect on the ascorbic acid content of orange beverage. Four levels were chosen, these being 0, 8, 16, 24 ppm corresponding to 0, 1, 2, 3 iodine tablets per liter of water. Figure 1 shows the results of those determinations. As can be seen, there is no clear-cut effect of iodine concentration on loss of ascorbic acid from orange beverage. Part of the problem stems from the fact that the variations in content over time are so slight that differences may be attributed to experimental error. Also, kinetic zero time differences would be hard to determine due to the large excess of ascorbate over iodine. Iodine ranged from 0 - 24 ppm which is, assuming a molecular

weight for iodine of 253.8 mg/mole, 0 - 94.5 μ mole/L of free available iodine. Ascorbate on the other hand is calculated to be at the initial concentration of 210 mg/L or 1192.5 μ mole/L. A slight decrease in measured kinetic zero time concentration can be noted from 224 mg/L with no iodine to 209 mg/L ascorbate with three tablets of iodine which might be attributed to loss by reaction with iodine. However, after this point (kinetic zero time), the rate of loss over the two hour period of the experiment is insignificant from a practical point of view. One 250 mL serving (8 oz) of the beverage, kept up to 2 h will still supply at least 85 % of a day's requirement of vitamin C, the requirement being 60 mg/day.

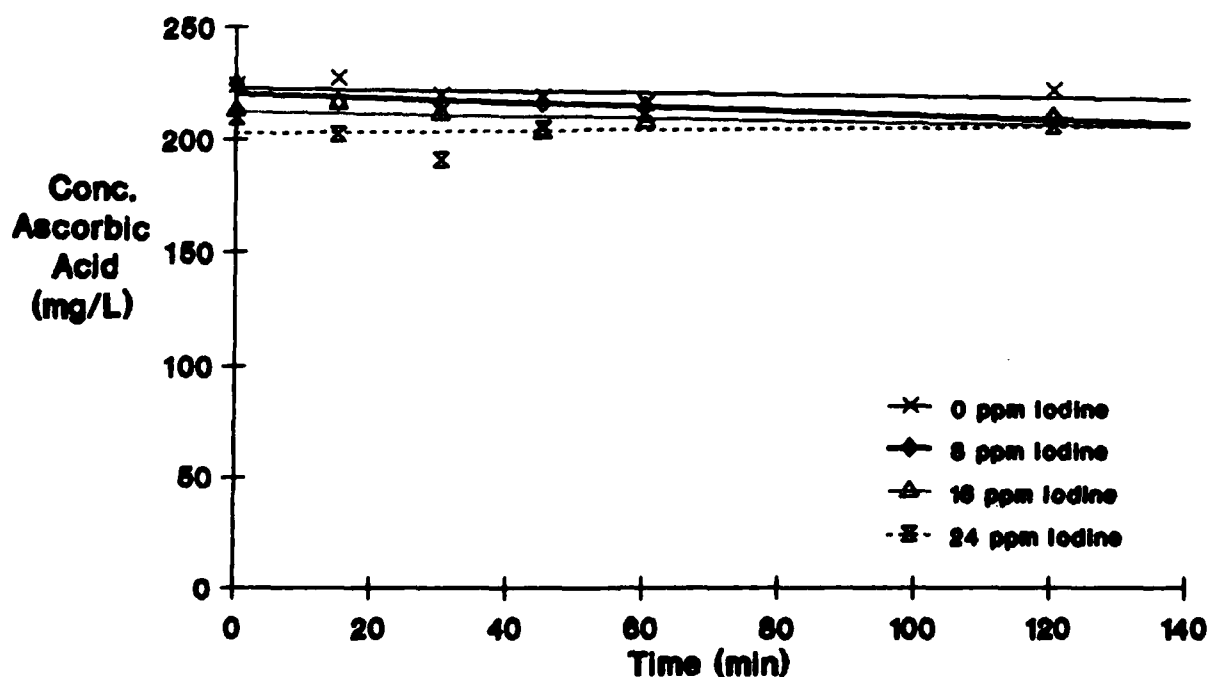


Figure 1. Effect of Iodine concentration on ascorbic acid content of orange beverage. Lines are plotted using linear regression. Slopes are: 0 ppm, -0.04 mg/L/min; 8 ppm, -0.11 mg/L/min; 16 ppm, -0.06 mg/L/min; 24 ppm, 0.02 mg/L/min.

Iodine concentration was also determined in each of the above four cases. It was found that iodine was immediately removed from solution by orange beverage powder (i.e., in each case by the 2 min initial sample the iodine was at 0 ppm).

A concern in the field is the effect of residual beverage in the canteen on the purification of nonpotable water added to that canteen. An experiment was run to determine the effect of orange beverage that could be left in a canteen. Table 6 gives the results of that determination. The equation obtained from a linear regression (correlation coefficient = 0.99) is as follows:

$$\% I_2 \text{ remaining} = 97.3 - 22.78 \times (\% OB)$$

where % I_2 is percentage of the original 16 ppm and % OB is vol/vol percentage of orange beverage to water. Using two tablets per quart canteen, one would assume an iodine concentration of around 16 ppm. However, this can be reduced to 8 ppm by 2.08 % (or 20 mL) orange beverage remaining in the canteen from a previous use. From this finding one can see that care must be taken to rinse a canteen between uses for maximum effectiveness and safe use of purification tablets.

For reconstituted orange beverage powder, kinetic runs were performed at 25° and 37°C. Determinations of thiamin and ascorbic acid concentrations were made in tap water and in water treated with iodine to yield an initial concentration of 16 ppm. The results of these experiments are shown in Figures 2 and 3. Linear regression was run for each case and half-life of the vitamin calculated for the conditions of the experiment. As can be seen, thiamin is essentially stable in orange beverage over a period of four hours at both 25° and 37°C. Ascorbic

acid, on the other hand, shows a definite decline with time, the half-life being approximately 300 min at 25°C and approximately 160 min at 37°C. The difference between tap water and iodinated water is negligible.

Table 6. Effect of Residual Orange Beverage on Iodine Content of Purified Water*

<u>% RESIDUAL ORANGE BEVERAGE ADDED</u>	<u>% IODINE REMAINING</u>
0.00	100.00
0.125	97.3
0.25	86.5
0.375	86.5
0.50	86.5
1.0	75.6
2.5	40.5

LINEAR REGRESSION

$$\% I_2 = 97.3 - 22.78 \times (\% OB); r = 0.99$$

% I₂ is percentage of original 16 ppm;

% OB is vol/vol % of orange beverage to water

*Measured amount of orange beverage added to 200 mL water containing 16 ppm iodine. Iodine content measured amperometrically after mixing with added orange beverage. Volumes and ppm converted to percentages based on 1 qt canteen and 16 ppm initial concentration iodine.

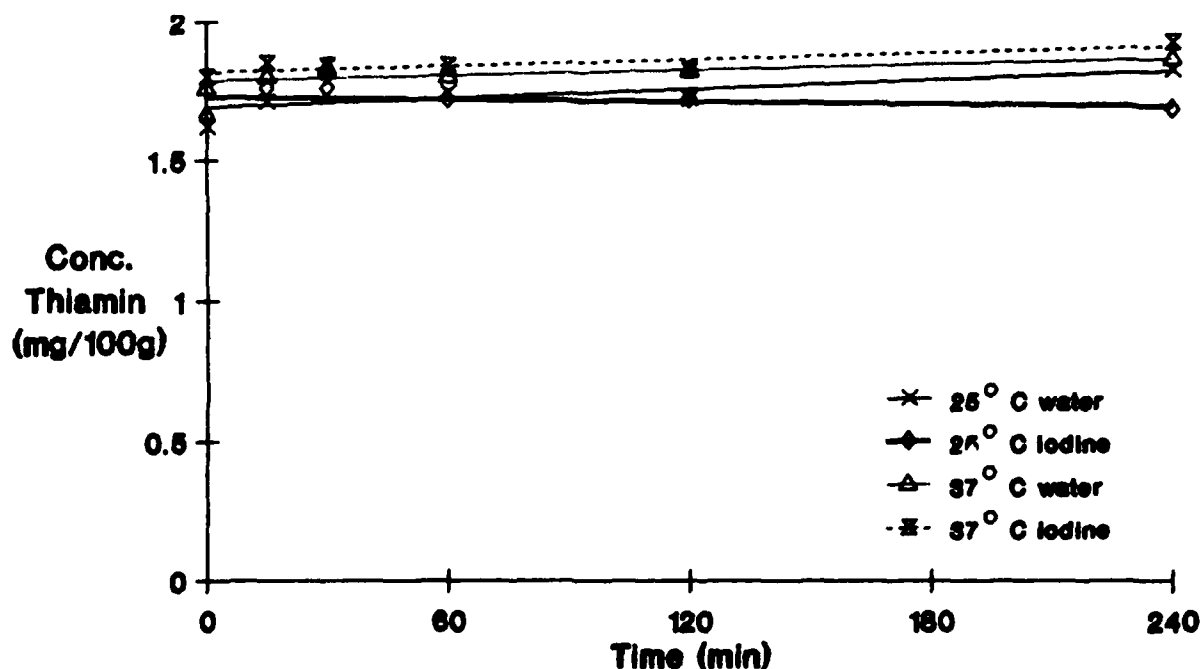


Figure 2. Effect of iodine on thiamin content of orange beverage at room and elevated temperatures.

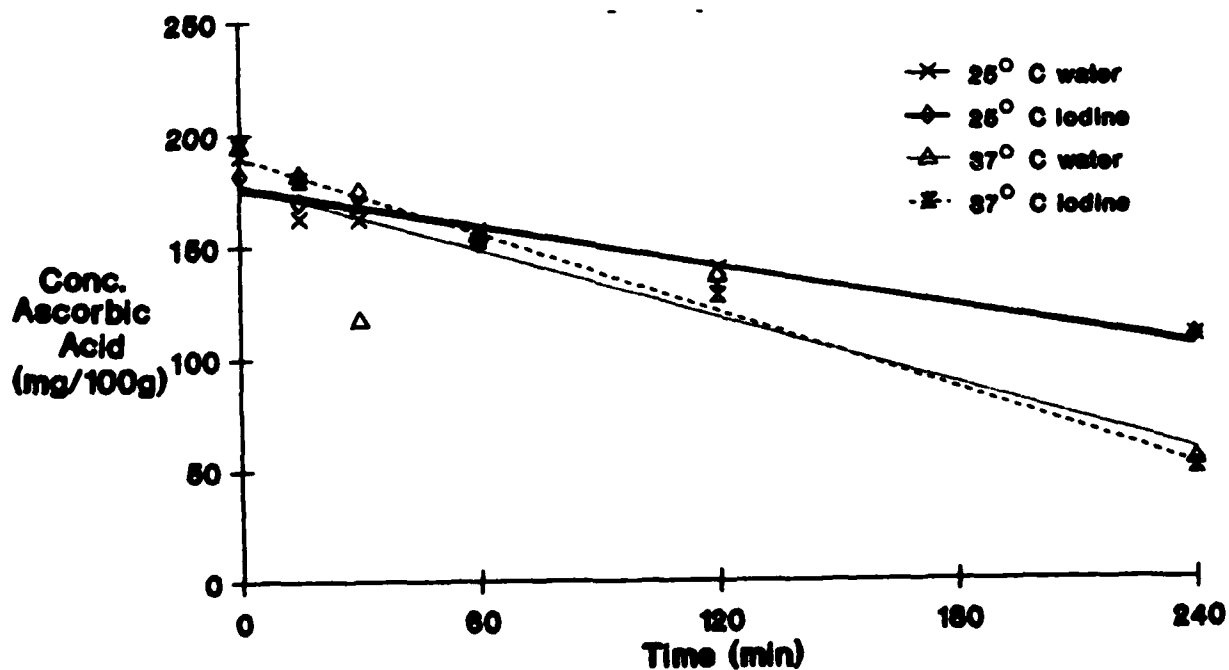


Figure 3. Effect of iodine on ascorbic acid content of orange beverage at room and elevated temperatures. At 25°C, half-life of ascorbic acid ($T_{1/2}$) in water = 302 mg/100g/min and in iodinated water = 295 mg/100g/min. At 37°C, $T_{1/2}$ in water = 178 mg/100g/min and in iodinated water = 164 mg/100g/min.

Orange beverage was reconstituted with hyperchlorinated water (4 - 5 ppm initial free available chlorine) and the ascorbic acid, thiamin and free available chlorine measured. It was found that chlorine did not affect either ascorbic acid or thiamin content over a period of time but was itself totally removed from solution (within the time required to take a sample and measure the concentration of chlorine).

2. Coffee. As mentioned earlier, coffee in field rations is fortified to a level of 15 mg ascorbic acid per 250 mL serving of coffee. Reference to Table 5 will show the importance of this item to vitamin C nutrition. It is most important in the Food Packet, Assault, providing 20 % of the daily intake of vitamin C.

The effects of iodine concentration on vitamin C in fortified coffee were measured following the same procedure as for orange beverage. As can be seen from comparison of Figure 1 and Figure 4, the results in this case are not so equivocal as in the case of orange beverage. A linear regression was run for each level of iodine present, and since all data points clustered closely together at each time interval, it was decided to run a linear regression using all data points. This gave a correlation coefficient of 0.96 and a regression equation of:

$$\text{vitamin C (mg/L)} = 53.34 - 0.397 \times \text{time}$$

and a half-life of vitamin C in coffee of 67 min at room temperature.

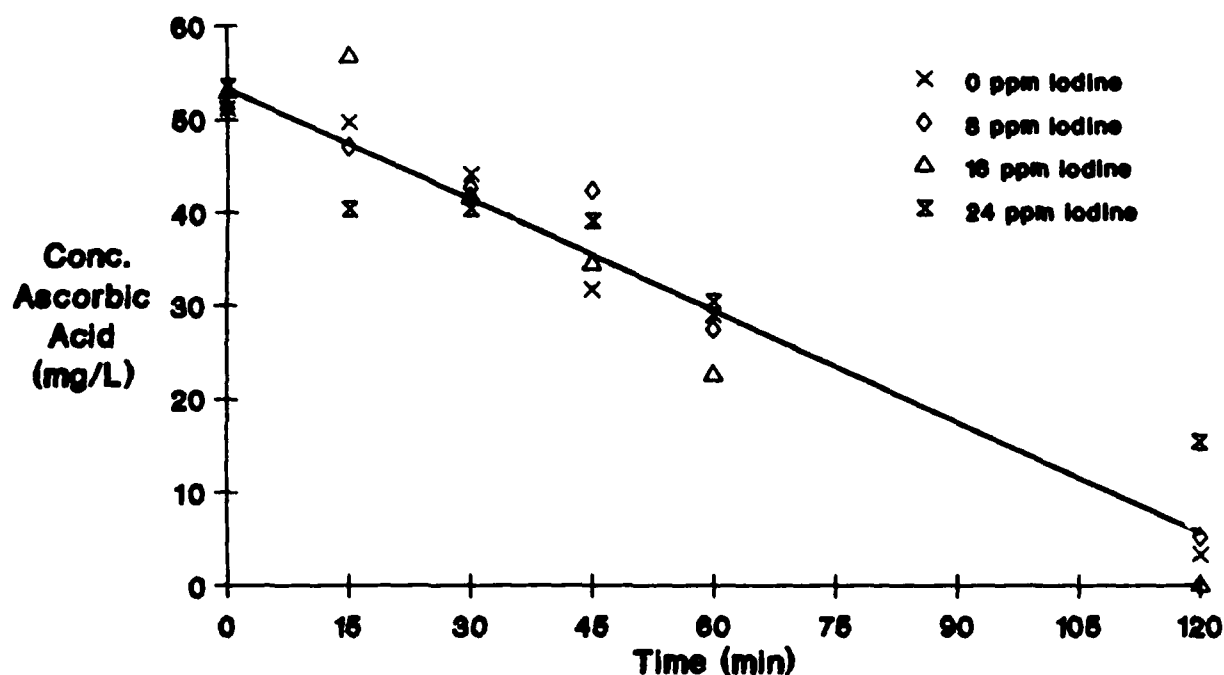


Figure 4. Effect of iodine concentration on ascorbic acid content in coffee. Regression line ($r = 0.96$); slope = -0.40 mg/L/min; Temp. = 20°C .

The results of the temperature dependence of vitamin C degradation with time in fortified coffee reconstituted with iodinated water are given in Figure 5. Fortified coffee was also reconstituted with hyperchlorinated water and these results are shown in Figure 6. The half-life of ascorbic acid in these solutions is not much different at 25° or 37°C , but at 60°C it appears that the half-life in iodinated water is double that in chlorinated water. In both cases there is very little ascorbic acid left after 20 min at normal hot coffee temperature of 60°C (less than 10 mg/L which is 2.5 mg/serving) as compared with the requirement of 60 mg/day.

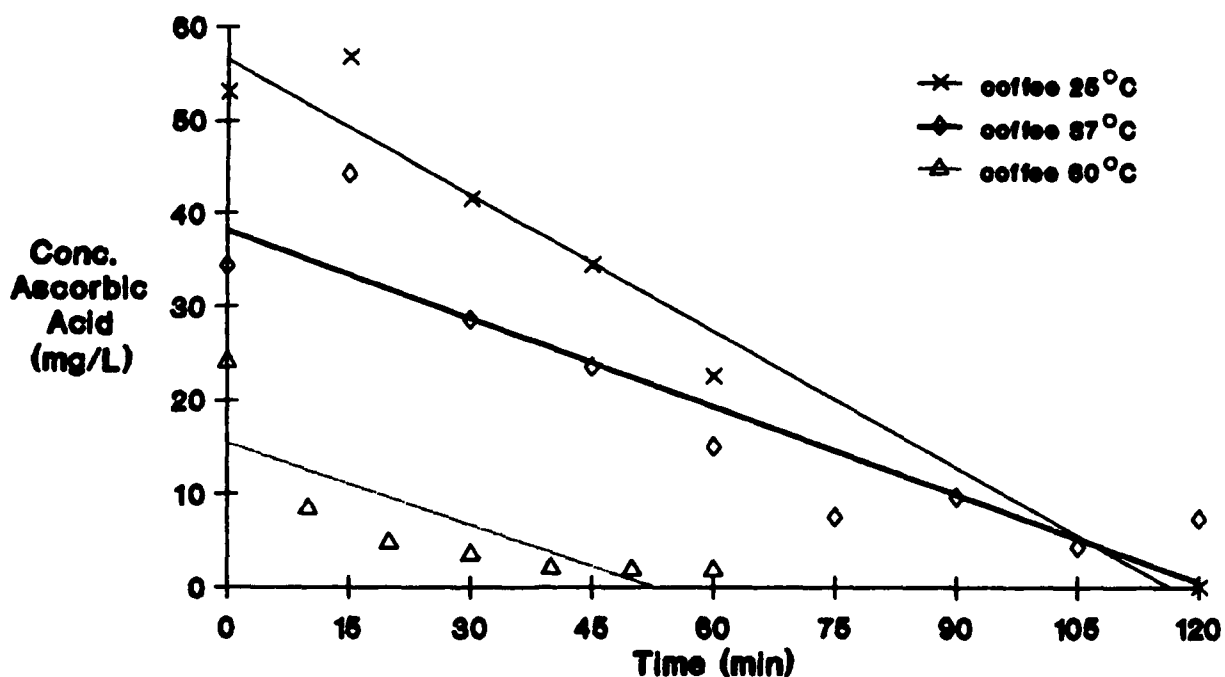


Figure 5. Vitamin C loss from coffee reconstituted with iodinated water (16 ppm iodine). Regression lines shown and half-lives calculated at each temperature. At 25°C, half-life = 58 min; at 37°C, half-life = 62 min; at 60°C, half-life = 27 min.

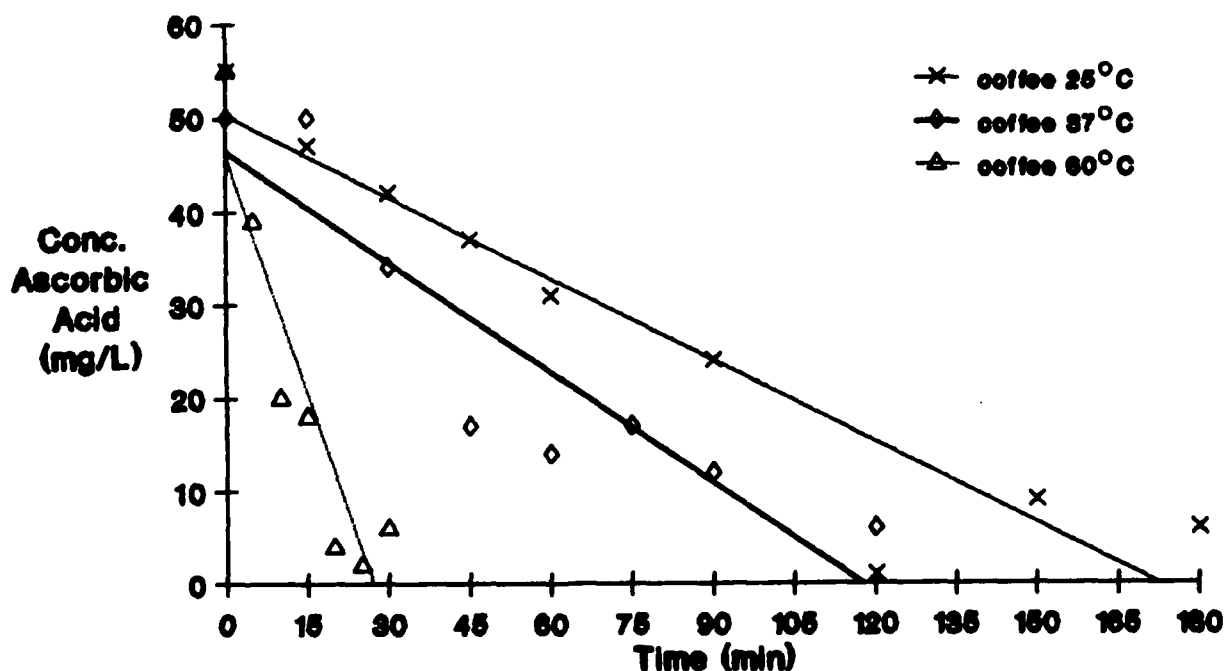


Figure 6. Vitamin C loss from coffee reconstituted with hyperchlorinated water. Regression lines shown and half-lives calculated at each temperature. At 25°C, half-life = 67 min; at 37°C, half-life = 59 min; at 60°C, half-life = 12 min.

In all cases, with both iodine and chlorine, initial aliquots were removed for halogen determination. It was found that both fortified and unfortified coffee rapidly removed all halogen present in the water used to reconstitute the coffee powder.

3. Electrolyte drinks. Electrolyte drink base was reconstituted using iodinated water containing 16 ppm free available iodine. The iodine content was followed over a period of two hours. The iodine content of the water control was measured initially and after two hours. The results of these determinations are shown in Figure 7. It should be noted that the time designated as zero time is in actuality two minutes after addition of water to powder. This time was necessary for complete mixing to occur. From Figure 7, it can be seen that mixing of the electrolyte beverage powder with the water results in a substantial reduction in iodine content. This effect is most pronounced with the flavored drink mixes where the flavoring acts to remove iodine, as has previously been shown to occur.^(5,7,8) In all cases, iodine values decrease with time. The citrus flavor, which contains reactive essential oils and terpenes, reacts most rapidly with iodine.

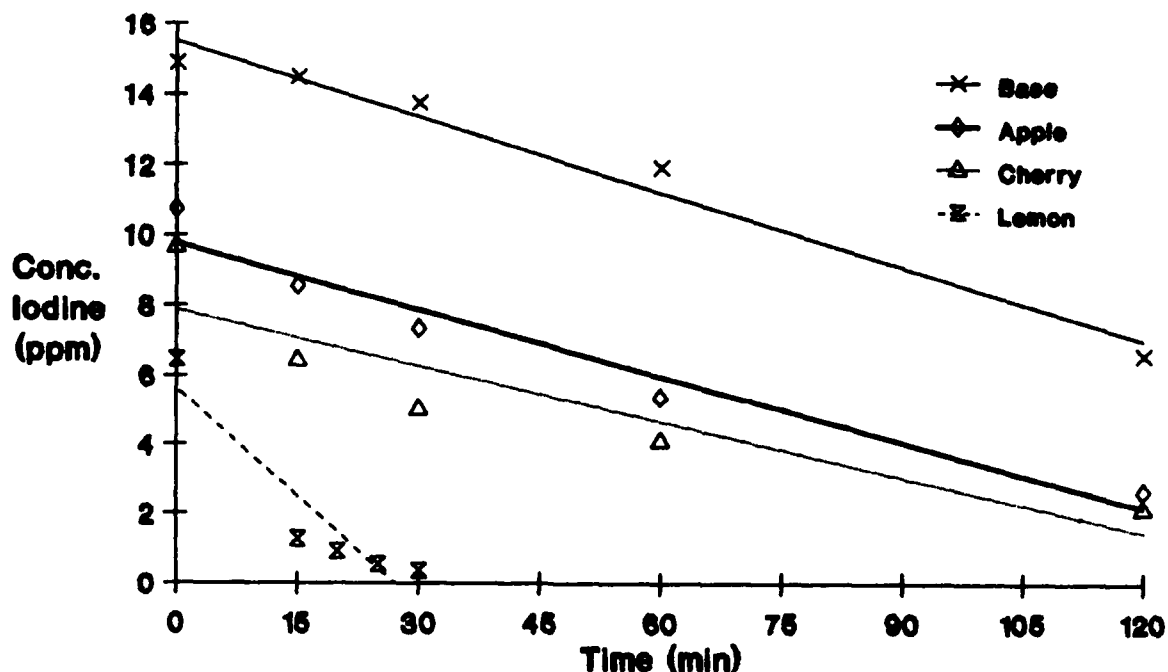


Figure 7. Iodine demand of electrolyte drink powders.
Temperature = 25°C. Regression lines shown.

To determine the effects of added vitamin C on the iodine demand of electrolyte drink base, 5 mg (28.4 μmole) of ascorbic acid were added to 750 mL drink base prepared with iodinated water whose iodine content had already been determined (14.32 ppm = 14.32 mg/L = 56.4 $\mu\text{mole/L}$). As quickly as possible the iodine content and the vitamin C content of the solution were measured. It was found that all of the vitamin C was destroyed immediately. The iodine content declined also, and after 3 min and 6 min was reduced to 3.94 ppm = 3.94 mg/L = 15.5 $\mu\text{mole/L}$. This is a reduction of 40.9 $\mu\text{mole/L}$ of iodine. Thus 37.9 $\mu\text{mole/L}$ of ascorbic acid removed 40.9 $\mu\text{mole/L}$ of iodine. This relationship appears to be one-for-one with one mole of ascorbic acid reacting with one mole of iodine. The reaction was so fast that the kinetics of decay of ascorbic acid could not be followed.

B. Neutral Beverages

Cocoa. Reference to Table 5 shows that cocoa powder is also an important contributor to vitamin C nutrition, especially in the Arctic Ration and the Food Packet, Assault. When reconstituted, cocoa beverage has a pH of 6.0 - 6.5 and therefore interactions between halogens and micronutrients might be different than in acid beverages.

Cocoa powder was reconstituted with iodinated water (initial concentration 16 ppm) and the iodine concentration determined. It was found that both fortified and unfortified cocoa immediately removed all iodine from solution at both room and elevated temperatures. Thiamin and ascorbic acid concentrations were followed in the reconstituted beverage powder at 25°, 37°, and 60°C over a period of time to determine the stability of these vitamins in the cocoa solutions. The results of these experiments are given in Figures 8 and 9. In each case linear regressions were run and half-lives of the vitamins were determined.

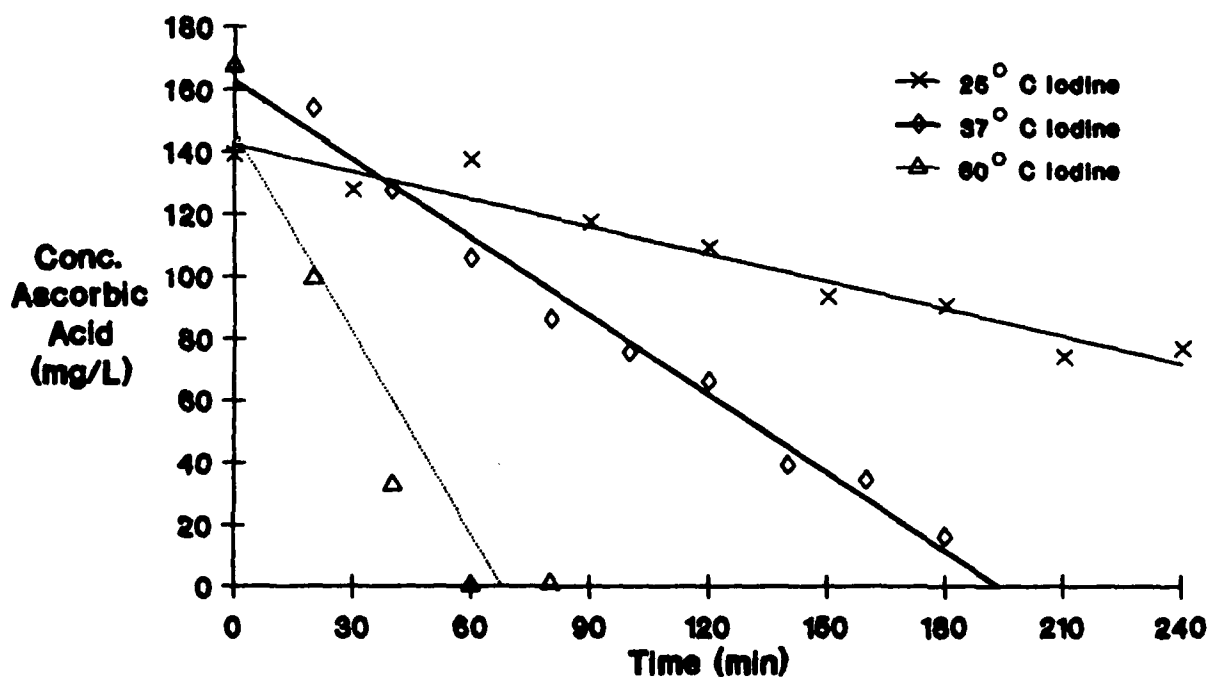


Figure 8. Vitamin C loss from cocoa reconstituted with iodinated water. Regression lines shown and half-lives calculated at each temperature. At 25°C, half-life = 246 min; at 37°C, half-life = 97 min; at 60°C, half-life = 28 min.

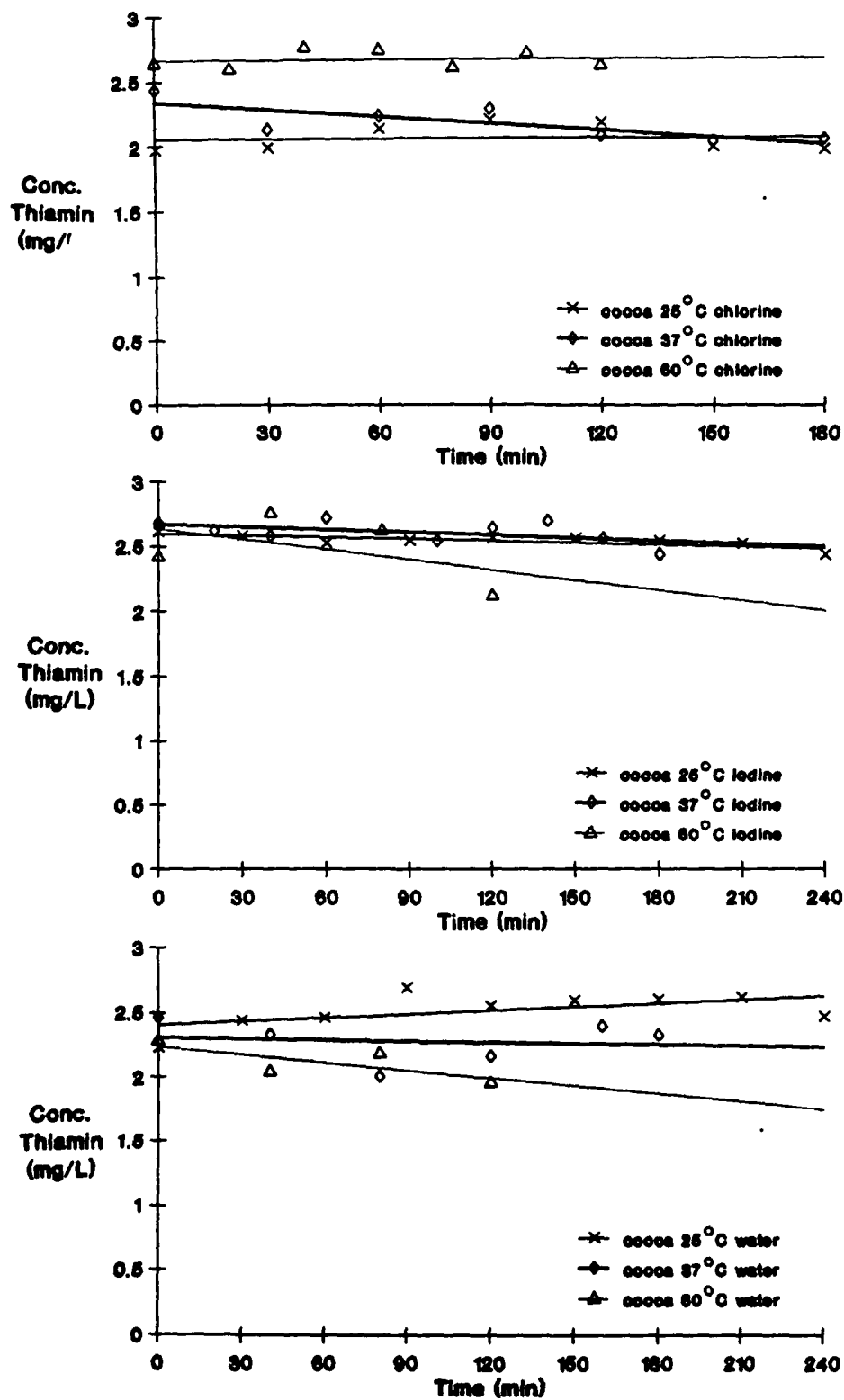


Figure 9. Effect of halogen and temperature on thiamin content of cocoa beverage.

Cocoa powder was also reconstituted with hyperchlorinated water and determinations made as above. Chlorine was immediately removed from solution by both fortified and unfortified cocoa. Results of time-temperature determinations for thiamin and ascorbic acid are given in Figures 9 and 10.

Reference to the figures shows that thiamin in cocoa appears to be unaffected by either temperature or halogen content of water for a period of at least two hours. As can be seen from Figure 11, ascorbic acid is lost over time in cocoa prepared with unhalogenated water. Higher losses are encountered at increased temperatures, regardless of type of water used to reconstitute. Note that at this pH (6.0 - 6.5) ascorbate is more sensitive to oxidation than at pH 4 - 6.⁽⁹⁾ However, Figures 12 to 14 show that at elevated temperatures, vitamin C has a longer half-life in cocoa reconstituted with hyperchlorinated water than with either tap or iodinated water. This apparent stabilization is observed despite the fact that in all cases there is no measurable halogen in the beverage at time zero of the determinations. A possible explanation for this stabilization could be reaction of chlorine with other ingredients in the cocoa to form a product more easily oxidized than the ascorbic acid in the beverage. By comparison with the vitamin C data expressed in Figures 5 and 6, this effect is the opposite of that noted in coffee.

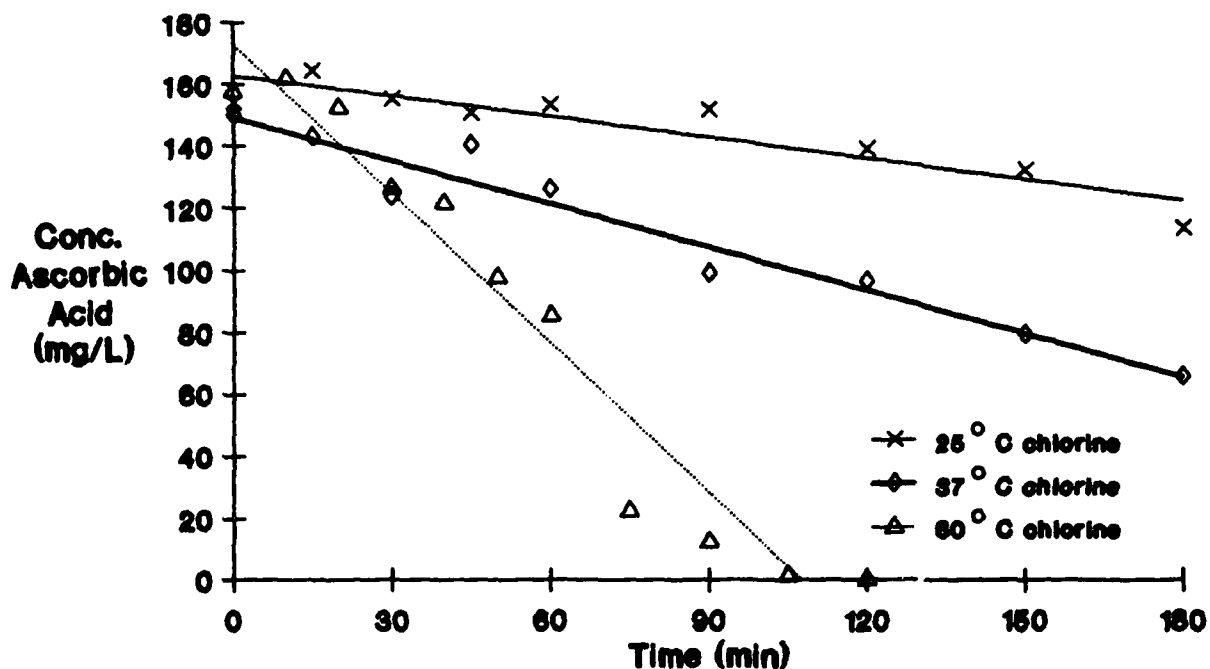


Figure 10. Vitamin C loss from cocoa reconstituted with hyperchlorinated water. Regression lines shown and half-lives calculated at each temperature. At 25°C, half-life = 354 min; at 37°C, half-life = 159 min; at 60°C, half-life = 51 min.

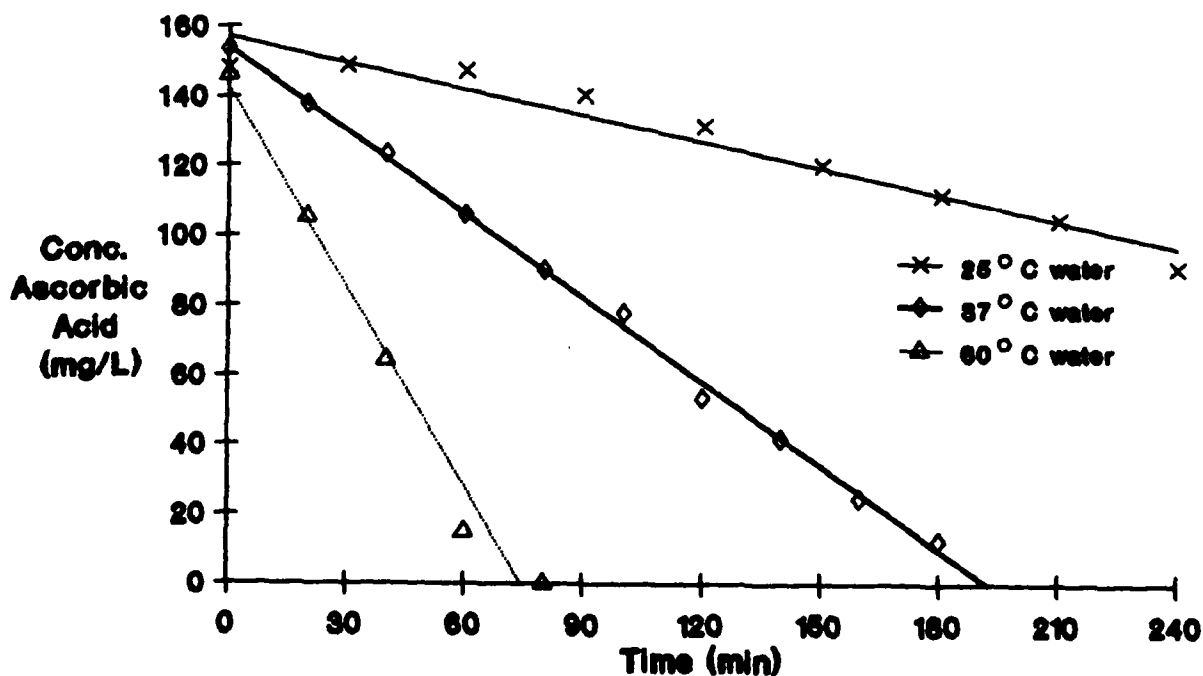


Figure 11. Vitamin C loss from cocoa reconstituted with tap water. Regression lines shown and half-lives calculated at each temperature. At 25°C, half-life = 314 min; at 37°C, half-life = 96 min; at 60°C, half-life 37 min.

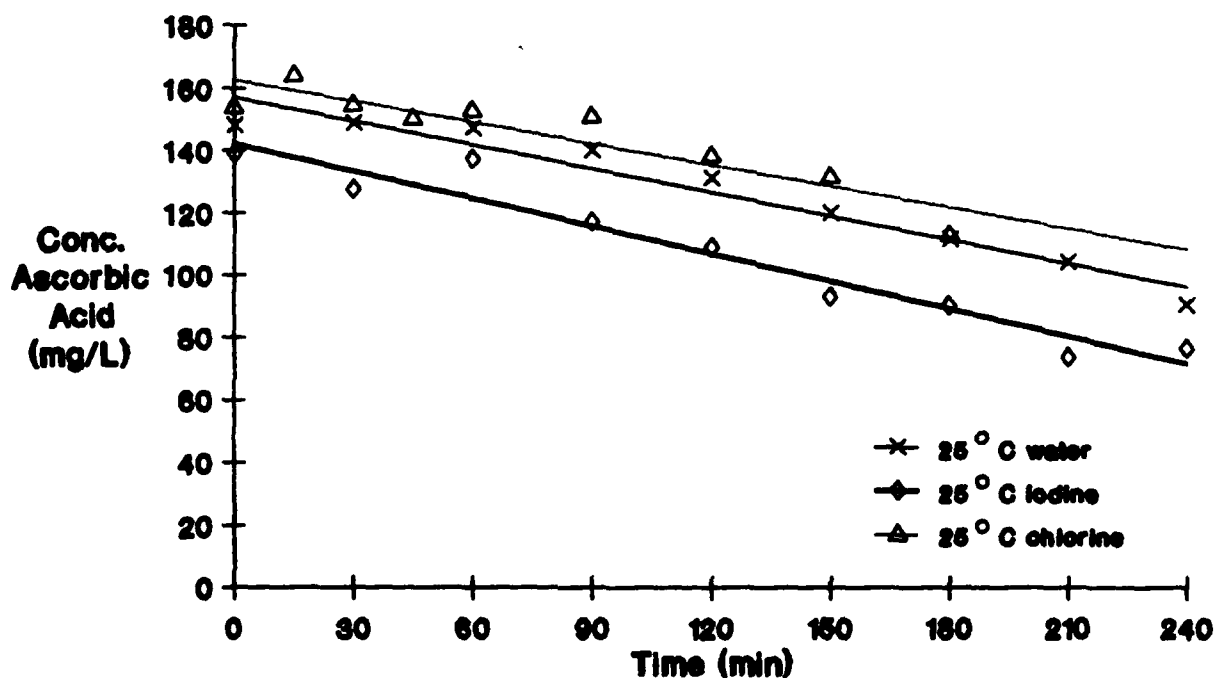


Figure 12. Comparison of vitamin C loss at 25°C from cocoa reconstituted with tap, iodinated, and hyperchlorinated waters. Regression lines shown. Half-lives calculated based on an initial concentration of 160 mg/L of vitamin C. In tap water, half-life = 320 min; in iodinated water, half-life = 276 min; in hyperchlorinated water, half-life = 348 min.

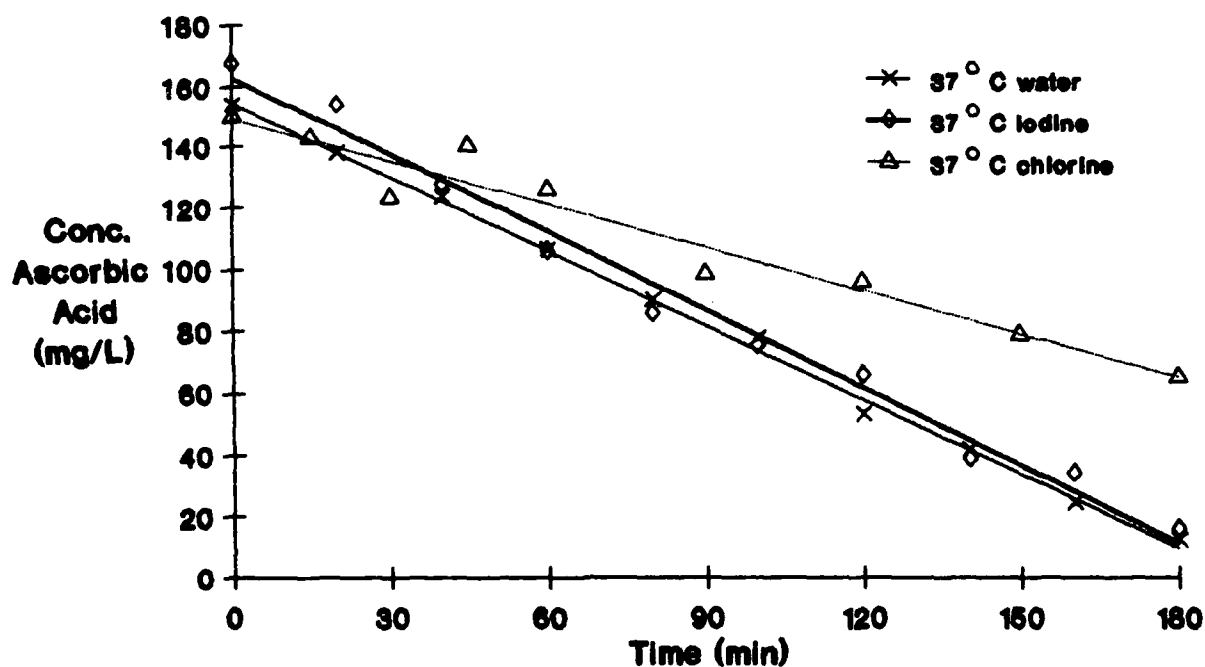


Figure 13. Comparison of vitamin C loss at 37°C from cocoa reconstituted with tap, iodinated, and hyperchlorinated waters. Regression lines shown. Half-lives calculated based on an initial concentration of 160 mg/L of vitamin C. In tap water, half-life = 100 min; in iodinated water, half-life = 95 min; in hyperchlorinated water, half-life = 170 min.

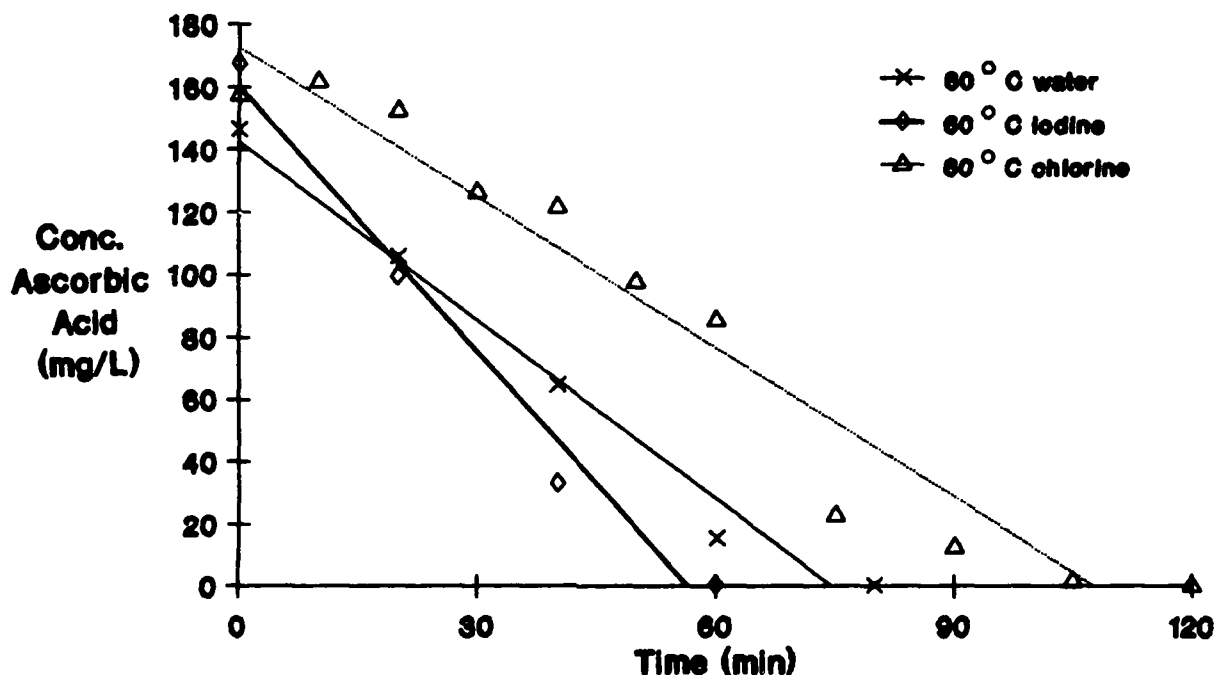


Figure 14. Comparison of vitamin C loss at 60°C from cocoa reconstituted with tap, iodinated and hyperchlorinated waters. Regression lines shown. Half-lives calculated based on an initial concentration of 160 mg/L of vitamin C. In tap water, half-life = 42 min; in iodinated water, half-life = 28 min; in hyperchlorinated water, half-life = 50 min.

C. Buffers.

1. Acid Buffer: pH = 3.5. As has previously been stated, several ingredients of the orange bar were tested for their iodine demand using pH 3.5 - 3.8 buffer. The most reactive ingredients, as can be seen from Table 2, are orange flavoring and ascorbic acid. Ascorbic acid immediately removed all iodine from the buffer solution. The reaction occurred too fast to be able to measure iodine concentrations. Figure 15 shows the decomposition rates of iodine in the presence of the orange flavorings, sucrose, glucose or thiamin. As can be seen, rate of decay of

iodine is the same in the buffer with thiamin (4 mg/L), glucose (3.5 g/L) or sucrose (102 g/L). Both of the orange flavorings, when tested at their normal use levels, did destroy iodine with a half-life of iodine of 15 min or less.

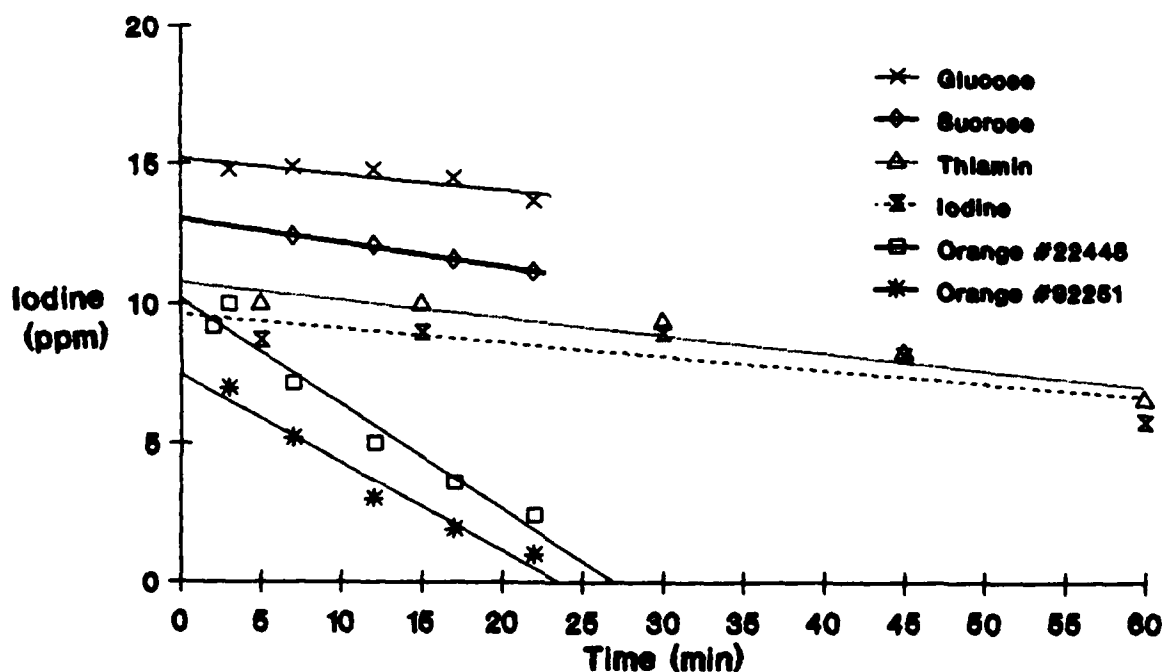


Figure 15. Loss of iodine from pH 3.7 buffer at room temperature in the presence and absence of ingredients. Regression lines shown and rates of loss calculated. Glucose (3.5 g/L): rate of loss = 5.5×10^{-2} ppm min⁻¹; sucrose (102 g/L): rate of loss = 8.2×10^{-2} ppm min⁻¹; thiamin (4 mg/L): rate of loss = 6.2×10^{-2} ppm min⁻¹; iodine (no other ingredients - 16 ppm nominal): rate of loss = 4.9×10^{-2} ppm min⁻¹; orange #22448: rate of loss = 3.8×10^{-2} ppm min⁻¹; orange #92251: rate of loss = 3.2×10^{-2} ppm min⁻¹.

2. Neutral buffer: pH = 7.0. Table 2 also lists those ingredients of cocoa beverage which were tested for reactivity with iodine (16 ppm nominal) in buffer solution. Cocoa, Cremora, and whey protein were the most reactive of the ingredients tested. A sample of each was tested for iodine at a maximum of five minutes after mixing, and in each case no iodine remained. Sodium chloride (1 g/L), cane sugar (105 g/L) and vanilla (2.5 mL extract/L) were also tested for compatibility with iodine. Figure 16 shows the iodine concentration vs time for these substances as well as for iodinated water and iodinated pH 7 buffer. It can be seen that sugar (105 g/L) slows the loss of iodine from buffer as does sodium chloride (1 g/L). On the other hand thiamin (4.8 mg/L), which was also tested, accelerates the removal of iodine from solution, the rate being approximately doubled.

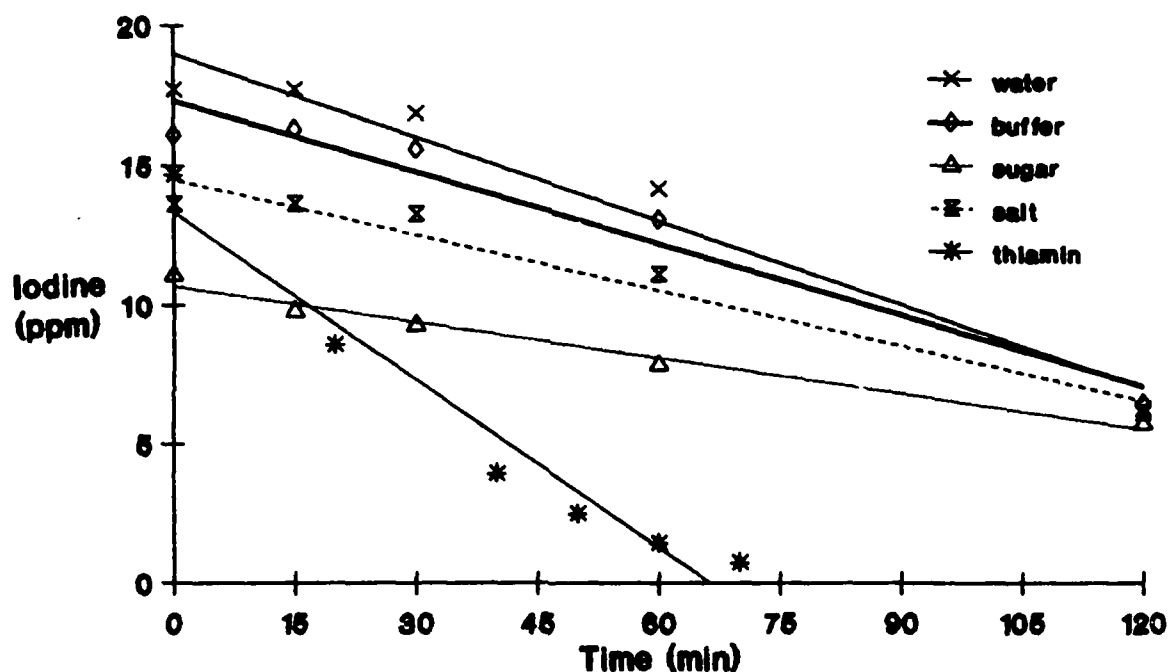


Figure 16. Iodine loss from water and pH 7.0 buffer at room temperature. Loss from buffer in the presence and absence of ingredients. Regression lines shown and rates calculated. Water: rate of loss = $1.0 \times 10^{-1} \text{ ppm min}^{-1}$; Buffer (no ingredients): rate of loss = $8.5 \times 10^{-2} \text{ ppm min}^{-1}$; Sugar (105 g/L): rate of loss = $4.3 \times 10^{-2} \text{ ppm min}^{-1}$; Salt (1 g/L): rate of loss = $6.6 \times 10^{-2} \text{ ppm min}^{-1}$; Thiamin (4.8 mg/L): rate of loss = $2.0 \times 10^{-1} \text{ ppm min}^{-1}$.

Both residual thiamin and iodine concentrations were converted to micromole/L ($\mu\text{M}/\text{L}$) and the results plotted vs time. Figure 17 gives the results of that plot. The results were also plotted semi-logarithmically to give Figure 18. From curve fitting operations, it was determined that the correlation was better for both reactions being logarithmic in reactant concentration. The equations obtained for loss of each reactant are

$$\text{Iodine: } y = 98.94 - 22.99 \ln x \quad r = 0.985$$

where $y = \text{time}$

$x = \text{iodine concentration remaining}$

$$\text{Thiamin: } y = 100.97 - 36.78 \ln x \quad r = 1.00$$

where $y = \text{time}$

$x = \text{thiamin concentration remaining}$

This would indicate that the loss of each reactant is a first order process.

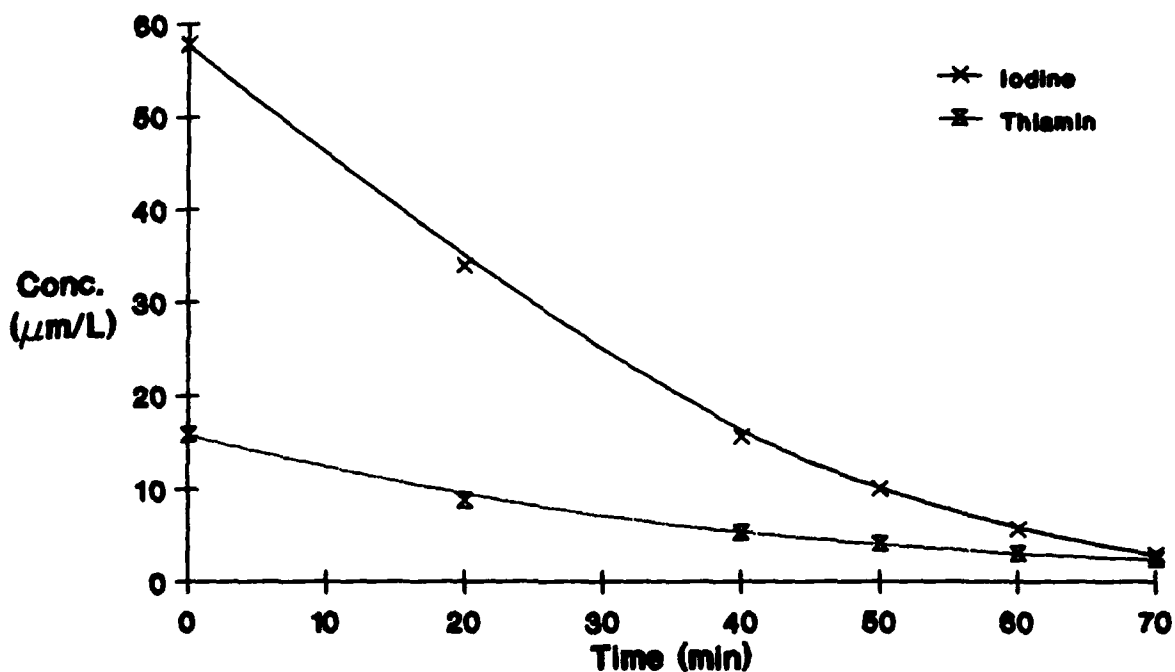


Figure 17. Loss of iodine and thiamin from pH 7.0 buffer at room temperature as a function of time.

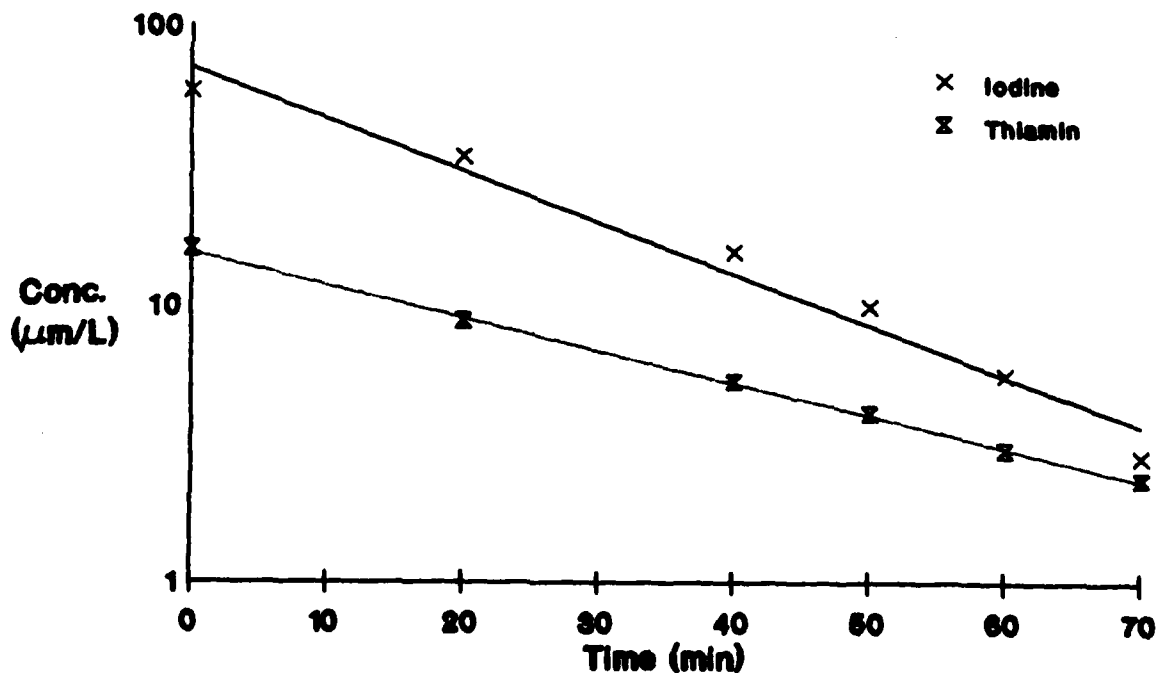


Figure 18. Semi-log plot of iodine and thiamin loss from pH 7.0 buffer at room temperature as a function of time. Regression lines shown. Iodine: $y = 98.94 - 22.99 \ln x$, where y = time and x = iodine concentration remaining; $r = 0.985$. Thiamin: $y = 100.97 - 36.78 \ln x$, where y = time and x = thiamin concentration remaining; $r = 1.00$.

A plot of concentration of iodine vs concentration of thiamin for each time yields a straight line of slope 4.16 as shown in Figure 19. This indicates a reaction of 4:1 = iodine to thiamin or one thiamin molecule can remove four iodine (I_2) molecules from solution. Thus, as little as 5 mg/L of thiamin can totally remove 16 ppm (or 2 tablets/L) of iodine in neutral solution. This amount of thiamin is slightly less than that found in fortified cocoa.

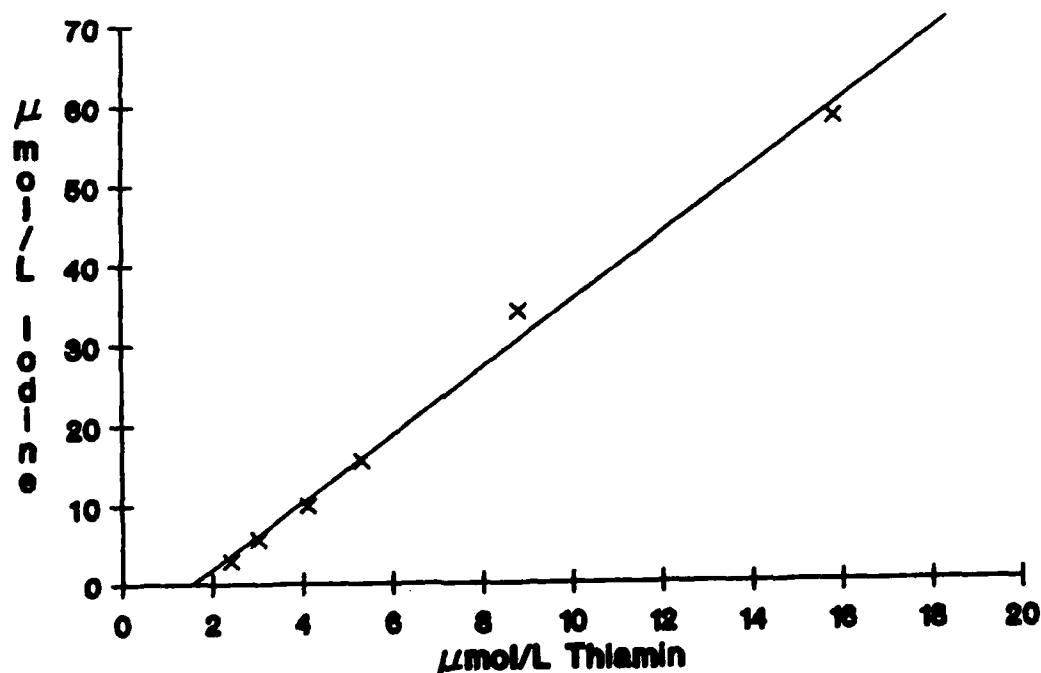


Figure 19. Plot of residual concentration of iodine vs residual concentration of thiamin in pH 7.0 buffer at room temperature over a 70 min time span. Regression line shown Slope = 4.16; $r = 0.995$.

D. Coated Vitamins and Potential Encapsulating Agents

It can be seen from Table 4 that encapsulating vitamin C with either water- or fat-soluble coatings does little to protect the iodine in solution. The capsules release the vitamin quickly at pH 3.8 and remove iodine within a short period of time. The only formulation that did not release vitamin C within minutes was the Durkee Durkote 145 - 50 which is 50% vitamin C. The vitamin C released into aqueous solution over a period of time and was essentially complete at 50 min at pH 3.5. At pH 7.0, the release proceeded slowly over a period of three hours and was not complete. Heating to 60°C (drinking temperature) fully released the vitamin.

The work done with iodine removal at pH 7, however, was rather equivocal. The first work showed iodine remaining in solution (4 ppm) 35 min after addition of coated vitamin C (Durkee C145 - 50; 21.2 mg Vit C per 200 mL). When one small capsule was crushed resulting in liberation of vitamin C, the iodine was immediately removed from the solution. However, a second kinetic determination showed complete removal of iodine from solution after 30 min as contrasted with data previously determined. It is postulated that the rate of removal of the iodine will depend on the integrity of the capsules. If the capsules have been damaged in any way, or if they may have faults, which are evident in photomicrographs (S. Cohen, Natick, personal communication), free ascorbic acid will be available to react with iodine and to remove it from solution.

Several potential encapsulating agents were tested for compatibility with iodine by visually observing changes in solution color. Some of these materials are in use commercially to produce encapsulated vitamins,

minerals, and flavorants. The results of the screening for compatibility with iodine are given in Table 3. As can be seen, several of these potential coatings have minimal iodine demand. Maltodextrin and apple pectin have good compatibility as water soluble agents, while Kaomel, a hydrogenated vegetable oil, is a good fat-soluble agent. Several of the potential encapsulating agents show reactivity at elevated temperatures so would not be useful for hot beverage formulations. Of the potential agents tested, maltodextrin is used in commercial formulations of coated vitamin C (Table 4). The loss of iodine with the commercial formulation is due to the rapid release of vitamin C and not reaction of the coating with iodine. To prevent release of vitamin C, a coating which is not water soluble would be necessary. For a useful formulation, the coating should also have minimal iodine demand.

CONCLUSIONS AND RECOMMENDATIONS

Both acid and neutral beverage formulations react to remove halogen from solution when individual portions are reconstituted with halogenated water. It does not matter whether the formulation is vitamin fortified or not, as other ingredients are extremely reactive toward iodine. Coffee, whether fortified or unfortified, will remove halogenating agent from solution as will cocoa. The electrolyte drink base removes little iodine, but addition of flavorings causes loss of iodine.

In buffer solutions, ascorbic acid reacted the fastest of all components tested at their normal use levels to remove iodine from solution, the reaction being instantaneous and too fast to measure. It is reasonable to assume that, in fortified beverages, the fastest reaction

will be that of ascorbic acid with iodine. Thus other vitamins, such as thiamin, will be protected from any reactions with iodine and the concentration of those vitamins will not change with time. Any change that occurs in other vitamins would be due to other causes and would not be affected by presence or absence of halogenating agent. As a matter of fact, in both cocoa and orange beverage, thiamin is not lost from the solution at room or elevated temperatures (Figures 2 and 9). Neither is the total level of ascorbic acid remaining affected by the presence of iodine. Since the vitamin is present in such large excess over iodine, the initial rapid drop in concentration is at the limit of detection for the assay. In the presence of hyperchlorinated water, however, at elevated temperatures (37° , 60°C) the rate of loss of ascorbic acid is slower than in the presence of iodinated water. This could be due to the presence of chlorinated reaction products, which may serve to protect the vitamin C from reacting by removing oxygen from the solution.

From a nutritional perspective, the problem of halogenating agents removing vitamins from reconstituted beverages is minimal. However, from a safety standpoint, if reconstituted beverages are stored in a canteen, it is imperative that the canteen be rinsed with water before a new batch of contaminated water is field purified with iodine tablets. As little as 20 mL, or less than 1 oz, of orange beverage remaining in the canteen can reduce the iodine concentration from 16 ppm to 8 ppm, which is borderline for purification purposes.

Encapsulation of vitamins at this time does not seem to offer a necessary or effective strategy to protect either vitamins or halogenating agents from interactions. Other ingredients present in beverages react to

remove halogenating agents in the absence of vitamins. Encapsulation does offer a useful technique to protect vitamins from interactions with other ingredients in beverage powder formulations, to minimize flavors imparted to beverage powder formulations by added vitamins, and to prolong the useful shelf life of such fortified beverages.

It would appear that the best way to assure microbiological safety of the drinking water when field water is used to make up ration beverages is to train troops to:

1. Rinse all residue from the canteen.
2. Follow strictly all directions for preparation of purified water, or alternatively to:

Find another method for field purification, such as

- a. Filtration, but it must be recognized that recontamination of filtered water is possible and supplemental addition of a germicide (iodine or chlorine) is now required by current medical doctrine.⁽¹⁰⁾

- b. Sterilization using an electrochemical ozonating unit, such as the Ster-O-Lyzer.⁽¹¹⁾ However, this technique requires electrical power; battery powered units are available.

One might also note that for highly educated and self-disciplined campers, addition of ascorbic acid has been advocated to remove the objectional taste of halogenated water.⁽¹²⁾ The self-discipline is needed because one must be sure sufficient time has been allowed for the halogens to be reacting with microbes in the water before such additions of ascorbate or any other flavor masker or flavorants are added.

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APPENDIX

ORANGE BEVERAGE (FORTIFIED)

<u>Ingredients</u>	<u>Percent by weight</u>
Sugar, granulated, extra fine	84.93 to 86.43
Citric acid, anhydrous (fine powder)	4.500
Dextrose (powder)	3.000
Tricalcium phosphate (anhydrous powder)	1.850
Glycerine (food grade)	1.000 to 1.500
Release agent*	0.600 to 1.200
Potassium citrate, anhydrous (fine granular)	0.820
Vitamin premix**	0.570
Xanthan gum	0.355
Silica	0.350
Natural orange flavor (equal to Fries and Fries No. 92251)	0.400
Natural and artificial orange flavor (equal to Fries and Fries No. 22448)	0.300
Cellulose gum	0.140
Titanium dioxide (water dispersible)	0.030
Artificial orange flavor (equal to McCormick No. F31129)	0.020
FD&C yellow No. 5 color	0.020
FD&C yellow No. 6 color	0.015

*See p. 43 for formulation

**See p. 43 for formulation

APPENDIX (CONTINUED)

RELEASE AGENT

<u>Ingredients</u>	<u>Percent by weight</u>
Soybean oil	80.00
Octaglycerol monooleate	10.00
Decaglycerol decaleate (sic)	10.00

VITAMIN PREMIX. Vitamin premix shall consist of the following ingredients and amounts per 170 mg (guaranteed): 1/

Ascorbic acid	60.00 mg <u>2/</u>
Niacinamide	0.50 mg
Pyridoxine hydrochloride	0.25 mg
Riboflavin, type S	0.45 mg
Thiamine (sic) mononitrate	0.30 mg <u>3/</u>
Vitamin E, 50 percent S.D. ^{4/}	4.50 mg
Vitamin D ₂ , 100 percent S.D.	2.00 mg (200 IU)
D-calcium pantothenate	2.00 mg
Sugar	<u>100.00 mg</u>
	170.00 mg

1/All ingredients in the vitamin premix mixture shall be food grade. The sugar shall have a granulation that aids in maintaining a stable mixture.

2/Encapsulated with malto dextrine (sic), 70 percent active, 30 percent coating.

3/Encapsulated with mono and di-glycerides, 33 percent active, 67 percent coating.

4/500 IU/gm of dl- α -tocopherol Acetate

APPENDIX (CONTINUED)

ELECTROLYTE DRINK

BASE

Fructose	15.00 g
FroDex 42(C)* (Maltodextrin)	10.00
Salt, NaCl	1.25
Potassium citrate	0.13
Citric acid, anhydrous	2.50
Tricalcium phosphate	0.25
Sodium Benzoate	0.20
Aspartame	<u>0.12</u>
	29.45

*42 dextrose equivalents (coarse)

APPENDIX (CONTINUED)

Lemon flavor

FM** lemon	0.30
No. 25,099-17	
FD&C yellow No.5	0.01

Cherry flavor

VD# cherry 594	.06
VD# cherry 556	.13
Stange cherry shade 001396	.004

Apple flavor

Hercules Apple Flavor	0.13
No. 6108710	
Caramel color powder	0.03

**Food Material Corporation (FM)
#Virginia Dare (VD)

COFFEE (MRE)

Added
ascorbic
acid

Type I, style 1,
composition 2
with ascorbic
acid

Average of not
less than 15
milligrams per
2-1/2 grams

APPENDIX (CONTINUED)

COCOA BEVERAGE POWDER (FORTIFIED - MRE)

Formulation. Type I finished product shall be formulated as follows:
Percentages shall be on a weight basis. (MIL-C-3031G dated 24 Jun 1981).

Sugar	Not more than 45 %
Cream substitute, dry (Cremora)	Not less than 35 %
Nonfat dry milk (solids) <u>1/</u>	Not less than 9.9 %
Cocoa	Not less than 9.5 %
Salt	Not more than 0.5 %
Vitamins <u>2/</u>	
Flavoring	Sufficient to provide an acceptable flavor in the prepared ready-to-use product

Lecithin (Optional ingredient)

Stabilizers (Optional ingredient)

1/Whey, dry, reduced lactose, may be substituted on a 1 for 1 basis.

2/Class 1 product shall have the following vitamin content per ounce:

Thiamine (sic) mononitrate - not less than 0.56 mg

Pyridoxine hydrochloride - not less than 0.84 mg

Vitamin A palmitate - not less than 1670 I.U.

Ascorbic acid - not less than 25 mg